

PTO CLAIMS/TJ

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~~Please cancel claims 1-57 and 68-74 without prejudice or disclaimer.~~

58. (Amended) A DNA construct comprising a sequence encoding SEQ ID NO: 71 operably linked to an expression vector.

59. A P45 protein produced from a cell containing a DNA construct as claimed in claim 58, wherein the protein is in monomeric, dimeric, or multimeric form.
60. A P45 protein as claimed in claim 59, wherein the cell is a bacterial cell.
61. A PEF complex comprising a P45 protein as claimed in claim 59.
62. An antibody that binds to a P45 protein as claimed in claim 59.
63. An antibody that binds to a PEF complex as claimed in claim 61.
64. A P45 protein produced from a cell containing a DNA construct as claimed in claim 58, wherein the P45 protein is produced as a fusion protein.
65. A P45 protein as claimed in claim 64, wherein the fusion protein comprises a calmodulin binding peptide.
66. A P45 protein as claimed in claim 65, wherein the expression vector is pCAL-n-EK.
67. A kit for replicating nucleic acids comprising at least one polymerase, a P45 protein as claimed in claim 59, and reagents for performing a polymerization reaction.
68. A kit as claimed in claim 67, wherein the P45 protein is present in a PEF complex.

75. A method for detecting the presence or absence of PEF activity in a sample comprising adding the sample to a nucleic acid polymerase reaction containing dUTP or dCTP and monitoring the inorganic pyrophosphate levels.

76. A method for detecting the presence or absence of PEF activity in a sample comprising adding the sample to a nucleic acid polymerase reaction containing dUTP and monitoring any change in polymerization levels.

77. A non-naturally occurring composition of matter comprising a P45 protein.

78. A composition of matter as claimed in claim 77, wherein the P45 protein is in monomeric, dimeric, or multimeric form.

79. A composition of matter as claimed in claim 77, wherein the P45 protein is present in a protein complex.

80. A composition of matter as claimed in claim 77, wherein the P45 protein is an analog P45 protein.

81. A method of producing a P45 protein comprising transferring a DNA construct as claimed in claim 58 into a host cell and expressing the P45 protein.

82. A method as claimed in claim 81, wherein the P45 protein is expressed as a fusion protein.

83. A method of producing a PEF analog protein comprising introducing at least one mutation into the sequence encoding P45 protein of the DNA construct of claim 58 or into a sequence encoding a dUTPase protein, transferring the sequence to a host cell, and expressing the PEF analog protein.

2 ✓ 84. (Amended) A DNA encoding a polypeptide possessing Polymerase Enhancing Factor (PEF) activity, the DNA comprising at least one of (a) one or more of SEQ ID NO: 32-35, 82, 83, or 70; (b) a sequence hybridizable to the complement of one or more those sequences under stringent conditions; and (c) a degenerate variant of (a).